

# Claims

- [c1] 1. A multi-layer column comprising:  
a chamber having a longitudinal axis, a first end including a first aperture through which a fluid sample is introduced into the chamber, a second end including a second aperture through which the fluid sample exits the chamber, and a cross-section generally transverse to the longitudinal axis; and  
a plurality of vertically stacked, microporous membrane layers stacked within the chamber, including at least a plurality of solid-phase substrates each carrying a different anti-analyte, each membrane layer being sufficiently porous to allow the fluid sample to flow through the membrane layer and to permit the sample fluid to flow through the plurality of vertically stacked membrane layers, each of the membrane layers being sized to occupy substantially all of the cross-section of the chamber.
- [c2] 2. The multi-layer column according to claim 1, wherein each of the membrane layers comprises a fiber matrix membrane.
- [c3] 3. The multi-layer column according to claim 1, wherein

the membrane layers are substantially transparent to light of at least a selected wavelength.

- [c4] 4. The multi-layer column according to claim 1, wherein the membrane layers are transparent to light.
- [c5] 5. The multi-layer column according to claim 1, wherein each of a plurality of the membrane layers comprises a capture layer carrying anti-analyte and at least one light shielding layer substantially coplanar with the capture layer.
- [c6] 6. The multi-layer column according to claim 5, wherein at least one of the light shielding layers includes a light absorption sub-layer.
- [c7] 7. The multi-layer column according to claim 5, wherein at least one of the light shielding layers includes a light reflection sub-layer.
- [c8] 8. The multi-layer column according to claim 1, wherein at least a plurality of the solid-phase substrates carry a blocking substance.
- [c9] 9. The multi-layer column according to claim 1, wherein substantially all surfaces within the chamber carry a blocking substance.
- [c10] 10. The multi-layer column according to claim 1,

wherein endmost membrane layers of the plurality of membrane layers are filter layers having pores sized to substantially prevent flow of particles of a preselected size to central membrane layers of the plurality of membrane layers.

- [c11] 11. The multi-layer column according to claim 1, wherein the membrane layers are axially spaced from one another along the longitudinal axis.
- [c12] 12. The multi-layer column according to claim 1, further comprising a buffer solution contained within the chamber.
- [c13] 13. The multi-layer column according to claim 1, further comprising a waste reservoir in fluid communication with the second aperture of the chamber.
- [c14] 14. The multi-layer column according to claim 1, further comprising a fluid flow port to provide a fluid exit flow path from within the chamber.
- [c15] 15. The multi-layer column according to claim 1, wherein each of the anti-analytes is selected from the group consisting of antibodies, antigens, ligand, ligand receptors, nucleic acids capable of hybridizing to an analyte nucleic acid, enzyme-linked immunosorbent analyte, proteins or fragments of proteins capable of form-

ing a complex with an analyte protein or protein fragment, and chemical compounds capable of having biological activity with a target analyte.

- [c16] 16. The multi-layer column of claim 1, wherein the membrane layers are substantially planar.
- [c17] 17. The multi-layer column according to claim 16, wherein the plane of each of the membrane layers are substantially perpendicular to the longitudinal axis of the chamber.
- [c18] 18. The multi-layer column according to claim 1, wherein the membrane layers are substantially planar, the planes of the membrane layers being oriented at an oblique angle with respect to the longitudinal axis.
- [c19] 19. A multi-layer column comprising:  
a chamber having a longitudinal axis, a wall that defines the chamber, a first end including a first aperture through which a fluid sample is introduced into the chamber, a second end including a second aperture through which the fluid sample exits the chamber; and  
a plurality of vertically stacked, microporous membrane layers stacked within the chamber, including at least a plurality of solid-phase substrates each carrying a different anti-analyte, each membrane layer being suffi-

ciently porous to allow a fluid sample to flow through the membrane layer and to permit the sample fluid to flow through the plurality of vertically stacked membrane layers, at least a plurality of the membrane layers engaging the wall of the chamber to inhibit the fluid sample from passing between the membrane layers and the wall of the chamber.

[c20] 20. A method of manufacturing a multi-layer column, comprising:  
stacking a plurality of sheets of solid support, at least some of the sheets being coated with an anti-analyte;  
cutting the plurality of sheets so that each of the sheets is sized to occupy substantially all of a cross-section of a chamber; and  
disposing the plurality of sheets in the chamber, whereby each of the sheets occupies substantially all of the cross-section of the chamber.

[c21] 21. A method of manufacturing a multi-layer column, comprising:  
stacking a plurality of sheets of solid support, at least some of the sheets being coated with an anti-analyte;  
cutting the plurality of sheets so that each of the sheets is sized to engage a wall of a chamber to inhibit a fluid sample from passing between the sheets and the wall of the chamber; and

disposing the plurality of sheets in the chamber,  
whereby each of the sheets engages the wall of the  
chamber.